

Fig. 1



NR-LU-13 Heavy chain variable region sequences

GAG GTT CAG CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA GTC AGG TTG TCC TGC
 Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys 22

CDR1

ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT ATG CAC TGG GTG ATA GAG AGG CCT GAA CAG GGC
 Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Met His Trp Val Ile Glu Arg Pro Glu Gln Gly 44

CDR2

CTG GAG TGG ATT GGA AGG ATT GAT CCT GCG AAT GGT AAT ACT AAA TGT GAC CCG AAG TTC CAG GGC
 Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Cys Asp Pro Lys Phe Gln Gly 66

AAG GCC ACT ATA ACA GCA GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT
 Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser 88

CDR3

GAG GAC ACT GCC GTC TAT TAC TGT TCT AGA GAG GTC CTA ACT GGG ACG TGG TCT TTG GAC TAC TGG
 Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg Glu Val Leu Thr Gly Thr Trp Ser Leu Asp Tyr Trp 110

GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA
 Gly Gln Gly Thr Ser Val Thr Val Ser Ser 120

NR-LU-13 Light chain variable region sequences

GAC ATC CAG ATG ATT CAG TCT CCA TCG TCC ATG TTT GCC TCT CTG GGA GAC AGA GTC AGT CTC TCT
 Asp Ile Gln Met Ile Gln Ser Pro Ser Ser Met Phe Ala Ser Leu Gly Asp Arg Val Ser Leu Ser 22

CDR1

TGT CGG GCT AGT CAG GGC ATT AGA GGT AAT TTA GAC TGG TAT CAG CAG AAA CCA GGT GGA ACT ATT
 Cys Arg Ala Ser Gln Gly Ile Arg Gly Asn Leu Asp Trp Tyr Gln Gln Lys Pro Gly Gly Thr Ile 44

CDR2

AAA CTC CTG ATC TAC TCC ACA TCC AAT TTA AAT TCT GGT GTC CCA TCA AGG TTC AGT GGC AGT GGG
 Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Asn Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly 66

TCT GGG TCA GAT TAT TCT CTC ACC ATC AGC AGC CTA GAC TCT GAA GAT TTT GCA GAC TAT TAC TGT
 Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Asp Ser Glu Asp Phe Ala Asp Tyr Tyr Cys 88

CDR3

CTA CAG CGT AAT GCG TAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA
 Leu Gln Arg Asn Ala Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 107

Fig. 2



Light Chain

1	5	10
ASP ILE GLN MET	THR GLN SER PRO SER	SER
11	15	20
LEU SER ALA SER	VAL GLY ASP ARG VAL	THR
21	25	30
ILE THR CYS ARG	ALA SER GLN GLY ILE	ARG
31	35	40
GLY ASN LEU ASP	TRP TYR GLN GLN LYS	PRO
41	45	50
GLY LYS GLY PRO	LYS LEU LEU ILE TYR	SER
51	55	60
THR SER ASN LEU	ASN SER GLY VAL PRO	SER
61	65	70
ARG PHE SER GLY	SER GLY SER GLY SER	ASP
71	75	80
TYR THR LEU THR	ILE SER SER LEU GLN	PRO
81	85	90
GLU ASP PHE ALA	THR TYR TYR CYS LEU	GLN
91	95	100
ARG ASN ALA TYR	PRO TYR THR PHE GLY	GLN
101	105	
GLY THR LYS LEU	GLU ILE LYS	

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.

Fig. 3



Heavy Chain

1	5	10
GLN VAL GLN LEU	VAL GLN SER GLY ALA	GLU
11	15	20
VAL LYS LYS PRO	GLY ALA SER VAL LYS	VAL
21	25	30
SER CYS LYS ALA	SER GLY PHE ASN ILE	LYS
31	35	40
ASP THR TYR MET	HIS TRP VAL ARG GLN	ALA
41	45	50
PRO GLY GLN GLY	LEU GLN TRP MET GLY	ARG
51	55	60
ILE ASP PRO ALA	ASN GLY ASN THR LYS	CYS
61	65	70
ASP LEU SER PHE	GLN GLY ARG VAL THR	ILE
71	75	80
THR ALA ASP THR	SER ILE ASN THR ALA	TYR
81	85	90
MET GLU LEU SER	SER LEU ARG SER ASP	ASP
91	95	100
THR ALA VAL TYR	TYR CYS SER ARG GLU	VAL
101	105	110
LEU THR GLY THR	TRP SER LEU ASP TYR	TRP
111	115	120
GLY GLN GLY THR	LEU VAL THR VAL SER	SER

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.



Alignment of the Light Chain Variable Regions of
NR-LU-13 (top) and humanized NRX451 (bottom).

```

DIQMISSPSSMFASLGDRVSLSC RASQGIRGNLD WYQKPGGTIKLLIY STSNLNS
.....
DIQMTQSPSSLSASVGDRTITC RASQGIRGNLD WYQKPQKGPGLLIY STSNLNS
                        CDR1                      CDR2
    
```

```

GVPSRFGSGSGSDYSLTISSLESEDFADYYC LQRNAYPYTF GGGTKLEIK
.....
GVPSRFGSGSGSDYTLTISSLQPEDFATYYC LQRNAYPYTF GGGTKLEIK
                        CDR3
    
```

Alignment of the Heavy Chain Variable Regions of
NR-LU-13 (top) and humanized NRX451 (bottom).

```

EVQLQQSGAELVKPGASVRLSCTASGFNIK DTYMH WVIERPEQGLEWIG
.....
QVQLVQSGAEVKKPGASVKVSCKASGFNIK DTYMH WVRQAPGQGLQWMG
                        CDR1
    
```

```

RIDPANGNTK CDPKFQGGKATITADTSSNTAYLQLSSLTSEDATVYYCS
.....
RIDPANGNTK CDLSFQGRVTITADTSINTAYMELSSLRSDDTAVYYCS
      CDR2
    
```

```

REVLTGTWSLDY WQGQTSVTVSS
.....
REVLTGTWSLDY WQGQTLTVSS
      CDR3
    
```

Fig. 5

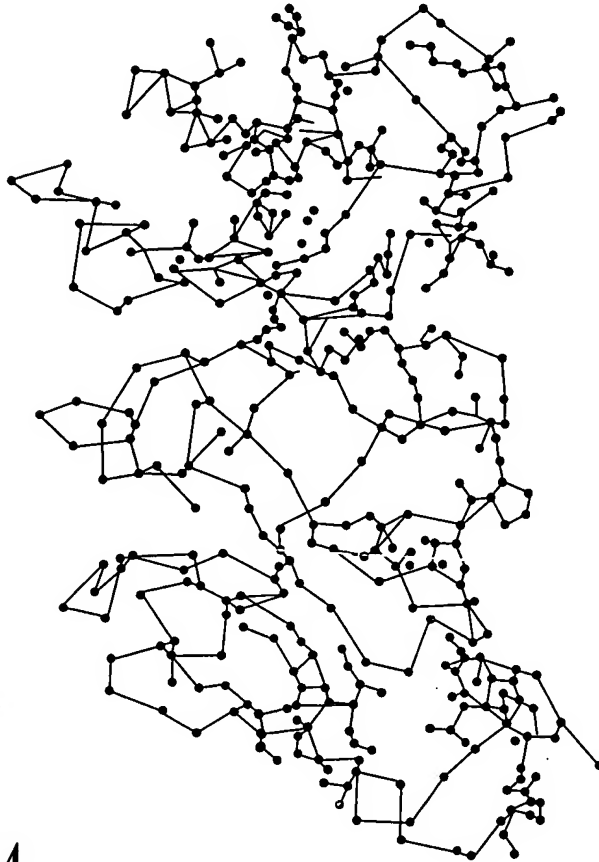


Fig. 6A

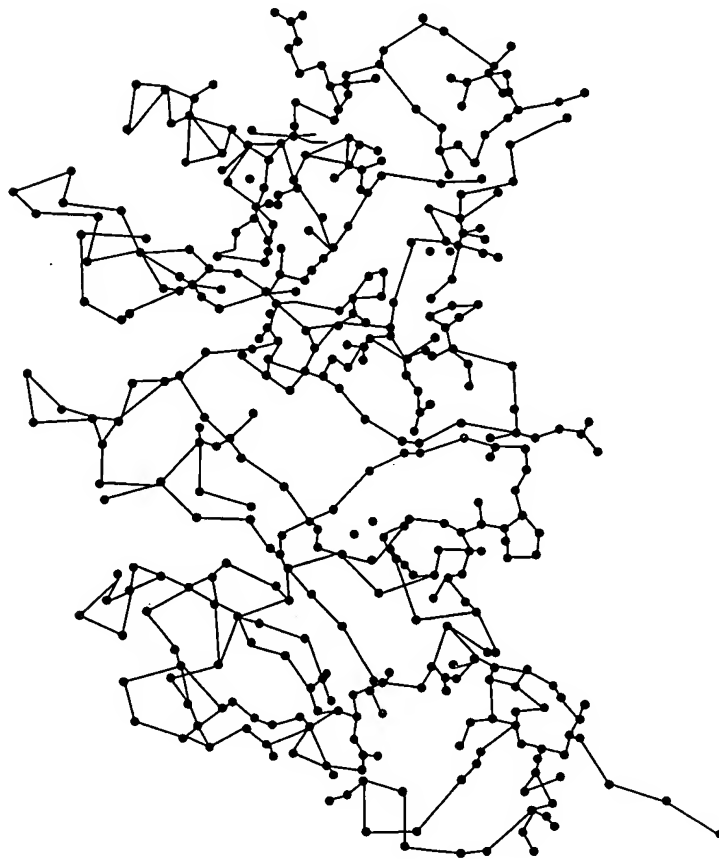


Fig. 6B

Same frequencies, but matching with human sequences. Only one occurrence of Asp at position 182 is found and no occurrences of Cys at position 181.



RES	181	182
A	-	0.48
R	-	0.02
N	0.01	0.18
D	0.00	0.00
C	0.00	0.00
Q	0.00	-
E	-	-
G	0.00	0.01
H	0.00	-
I	-	0.00
L	-	0.00
K	0.00	0.00
M	-	-
F	0.03	-
P	0.00	0.01
S	0.01	0.23
T	-	0.02
W	0.00	-
Y	0.91	-
V	0.00	0.02
X	0.01	0.02
O	-	-
-	-	-
Z	-	-
B	-	0.00
Total	1.00	1.00

Fig. 7A

Sequence positions 50 and 183 are structural mutations within 5 A of the CDR's. Frequency of residue types at these positions are identical.



Position 50 (153 human lambda sequences)

RES	50
A	-
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	-
K	-
M	0.00
F	-
P	0.93
S	-
T	-
W	-
Y	-
V	-
X	0.06
O	-
-	-
Z	-
B	-
Total	1.00

Fig. 7B



Position 50 (279 human kappa sequences)

RES	50
A	0.00
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	0.00
K	-
M	-
F	-
P	0.96
S	-
T	-
W	-
Y	-
V	-
X	0.03
O	-
-	-
Z	-
B	-
Total	1.00

Fig. 7C

Position 50 is highly conserved in all the sequences, but proline can be exchanged by Ile or Leu. The framework used for the light chain (6fab) does have an Ile at this position. If this position is compared to other structures the backbone torsions are the same for structures with a Pro and an Ile at this position.



Position 50 (153 human lambda sequences)

RES	183
A	0.06
R	-
N	0.00
D	0.21
C	-
Q	0.15
E	0.01
G	0.01
H	-
I	0.00
L	0.00
K	0.00
M	-
F	0.00
P	0.40
S	0.01
T	0.01
W	-
Y	0.00
V	0.08
X	0.02
O	-
-	-
Z	-
B	0.00
Total	1.00

Fig. 7D



Position 183 (1210 mouse sequences)

RES	183
A	0.16
R	0.00
N	0.00
D	0.13
C	-
Q	0.16
E	0.25
G	0.02
H	0.00
I	-
L	-
K	0.00
M	-
F	-
P	0.17
S	0.08
T	0.00
W	-
Y	-
V	0.00
X	0.02
O	-
-	-
Z	-
B	-
Total	1.00

Leu is seen in human sequences at this position, but never in murine sequences, for both human and murine Sequences P is the most frequently occurring residue at position 183.

Fig. 7E

Comments for pcDNA3:

5446 nucleotides

CMV promotor: bases 209-863

T7 promotor: bases 864-882

Polylinker: bases 889-994

Sp6 promotor: bases 999-1016

BGH poly A: bases 1018-1249

SV40 promotor: bases 1790-2115

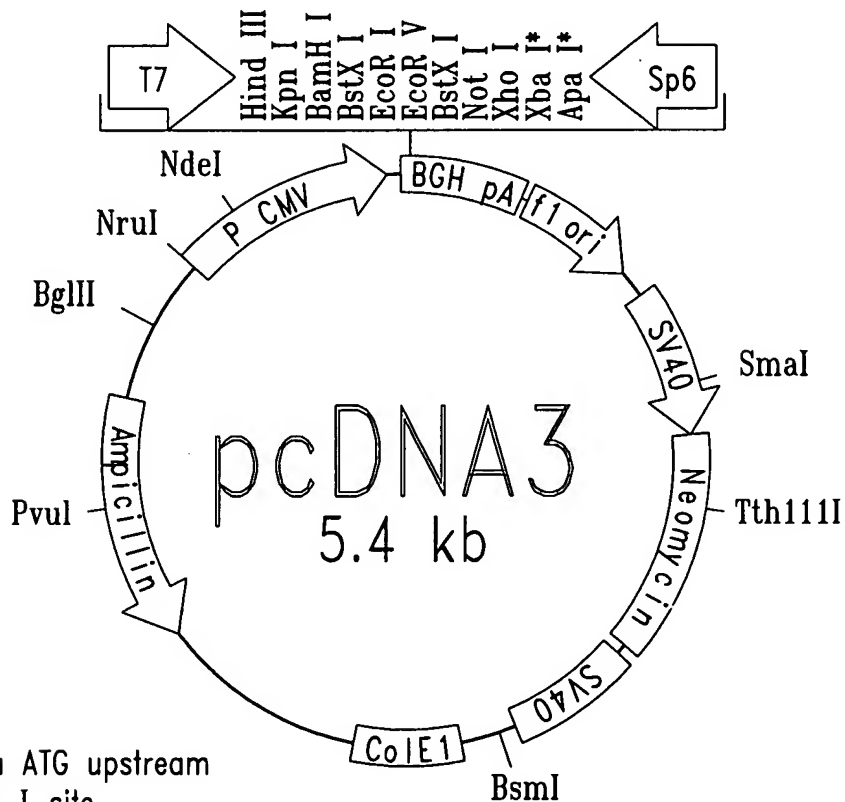
SV40 origin of replication: bases 1984-2069

Neomycin ORF: bases 2151-2945

SV40 poly A: bases 3000-3372

ColE1 origin: bases 3632-4305

Ampicillin ORF: bases 4450-5310



*There is a ATG upstream of the Xba I site

Fig. 8

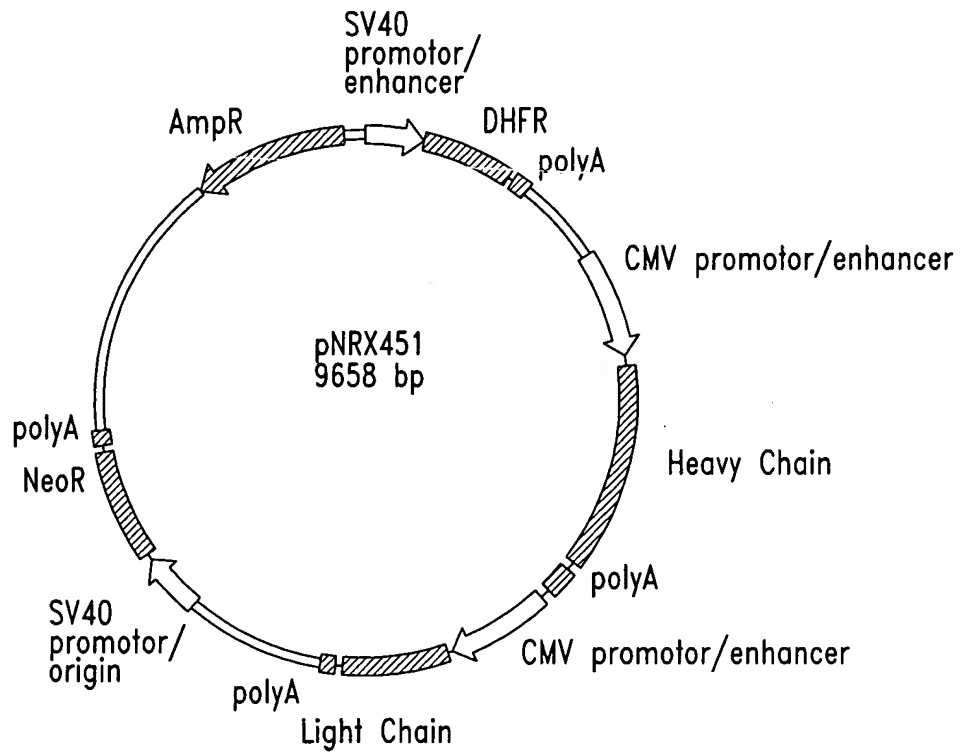


Fig. 9

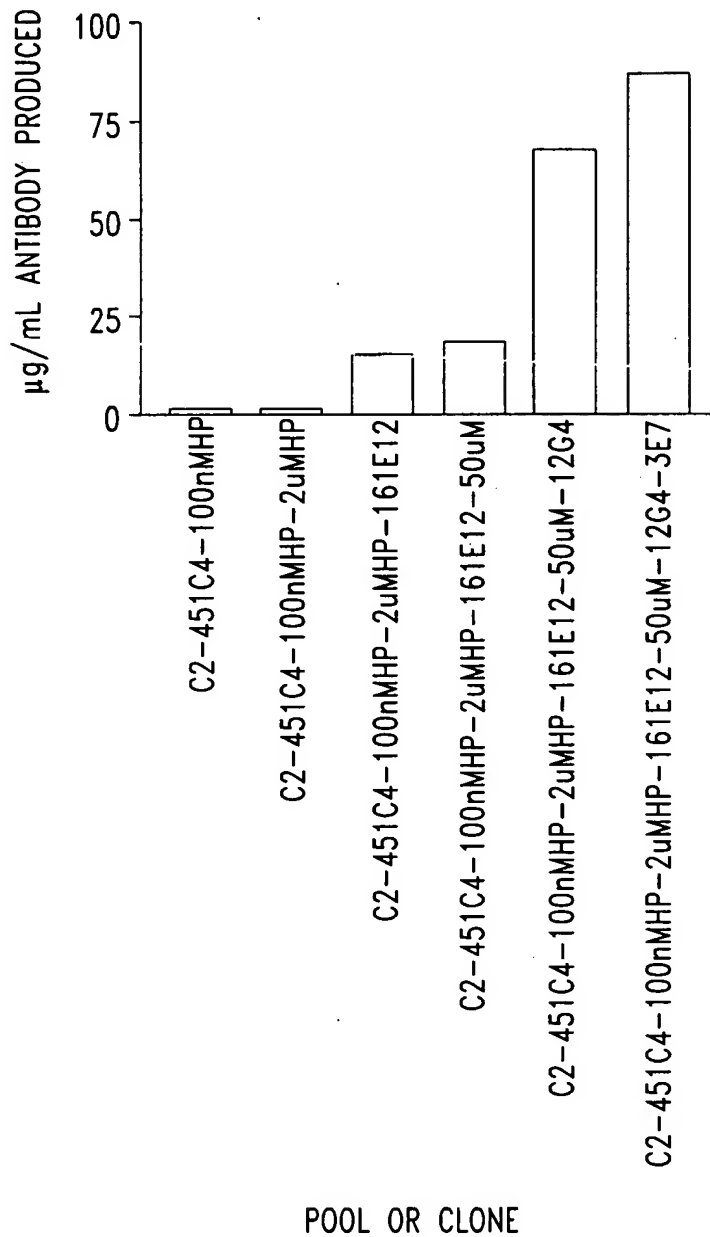


Fig. 10



COMPETITIVE IMMUNOREACTIVITY

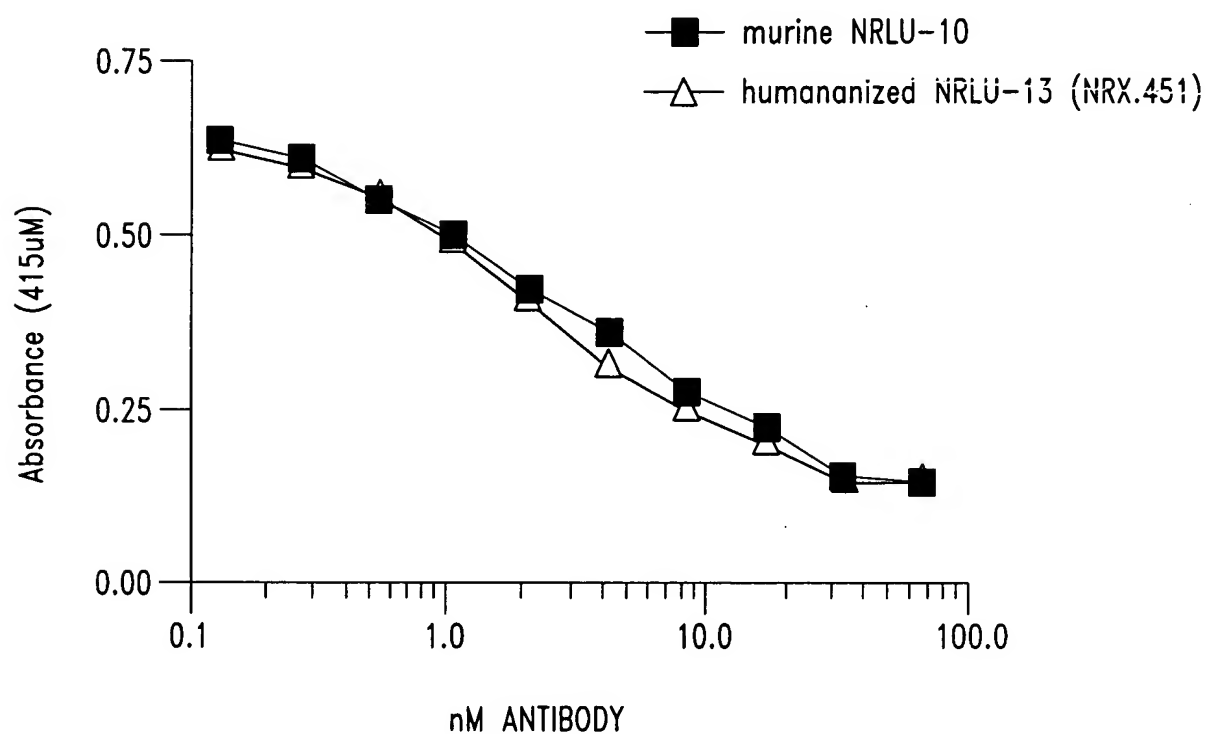


Fig. 11

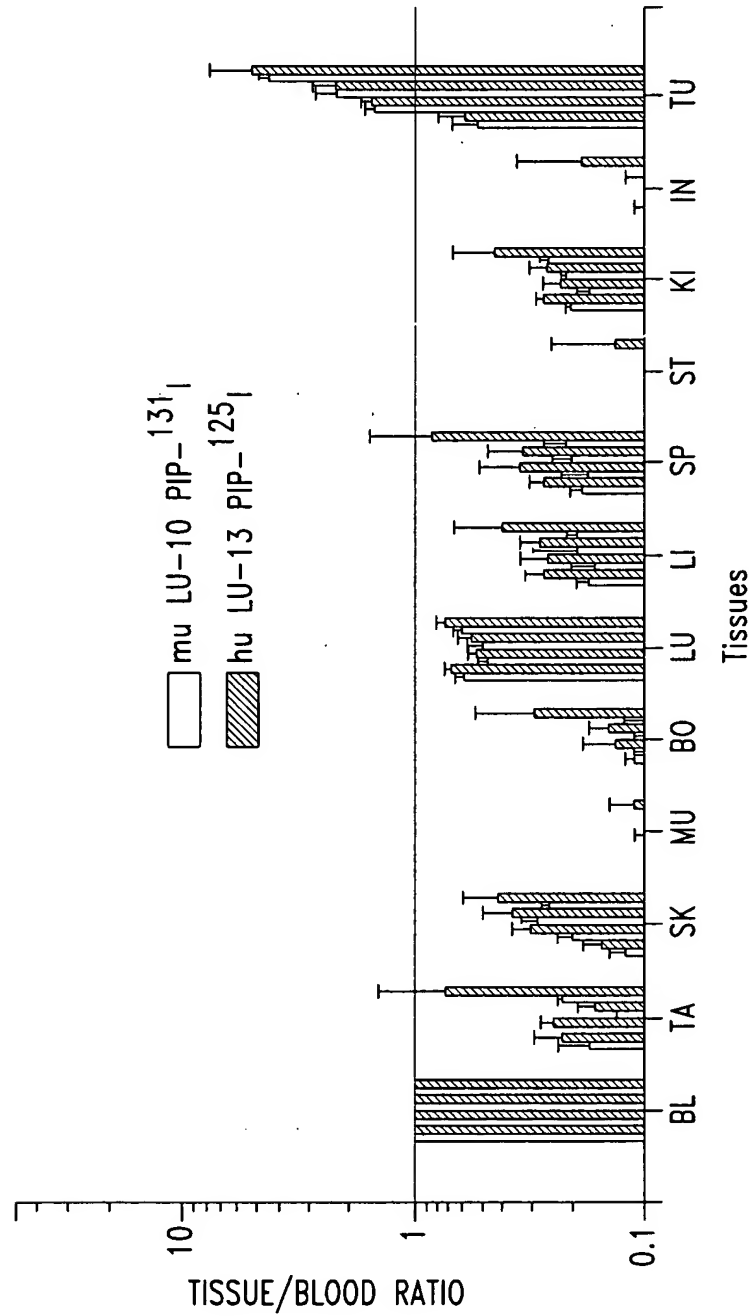


Fig. 12

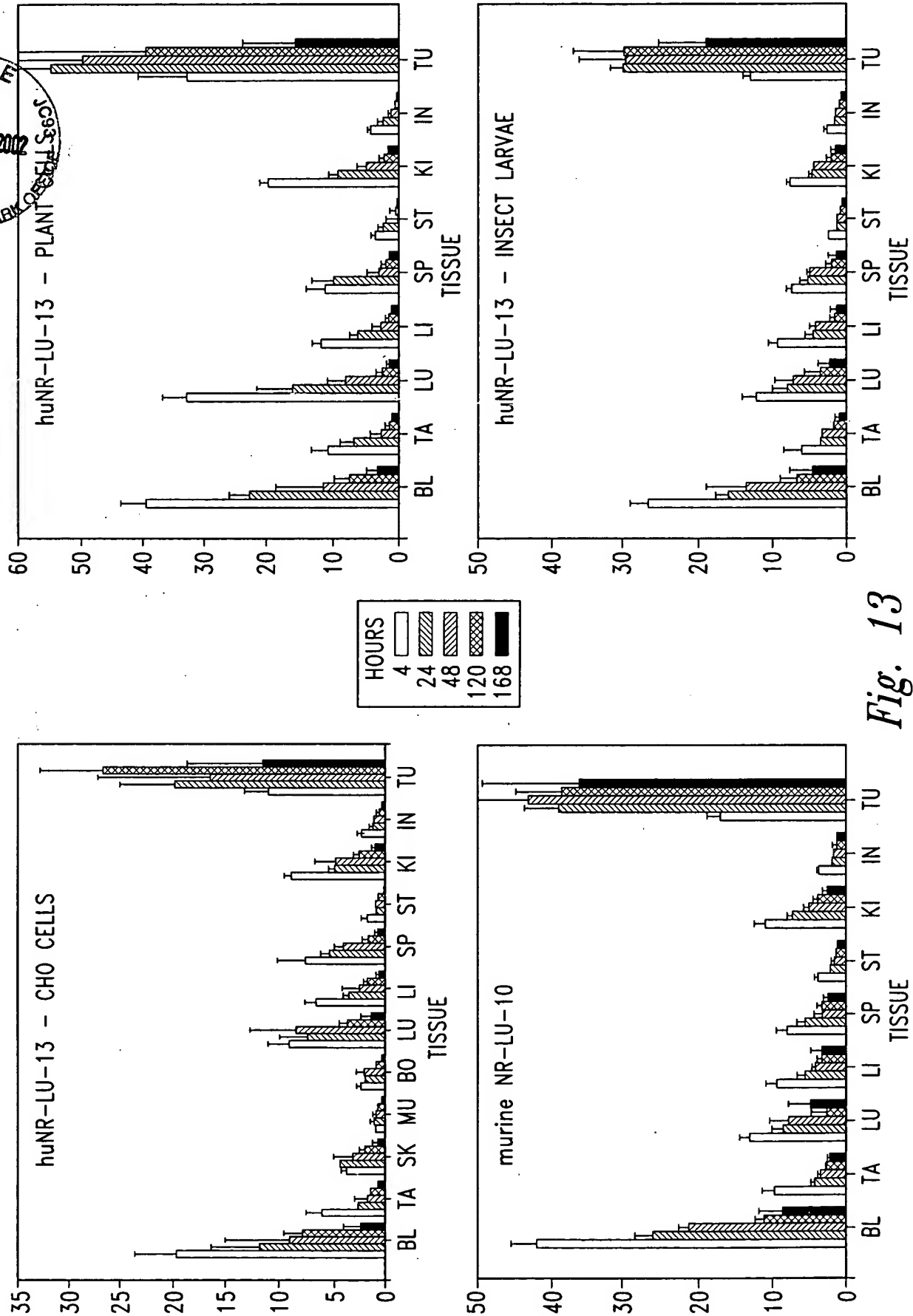


Fig. 13

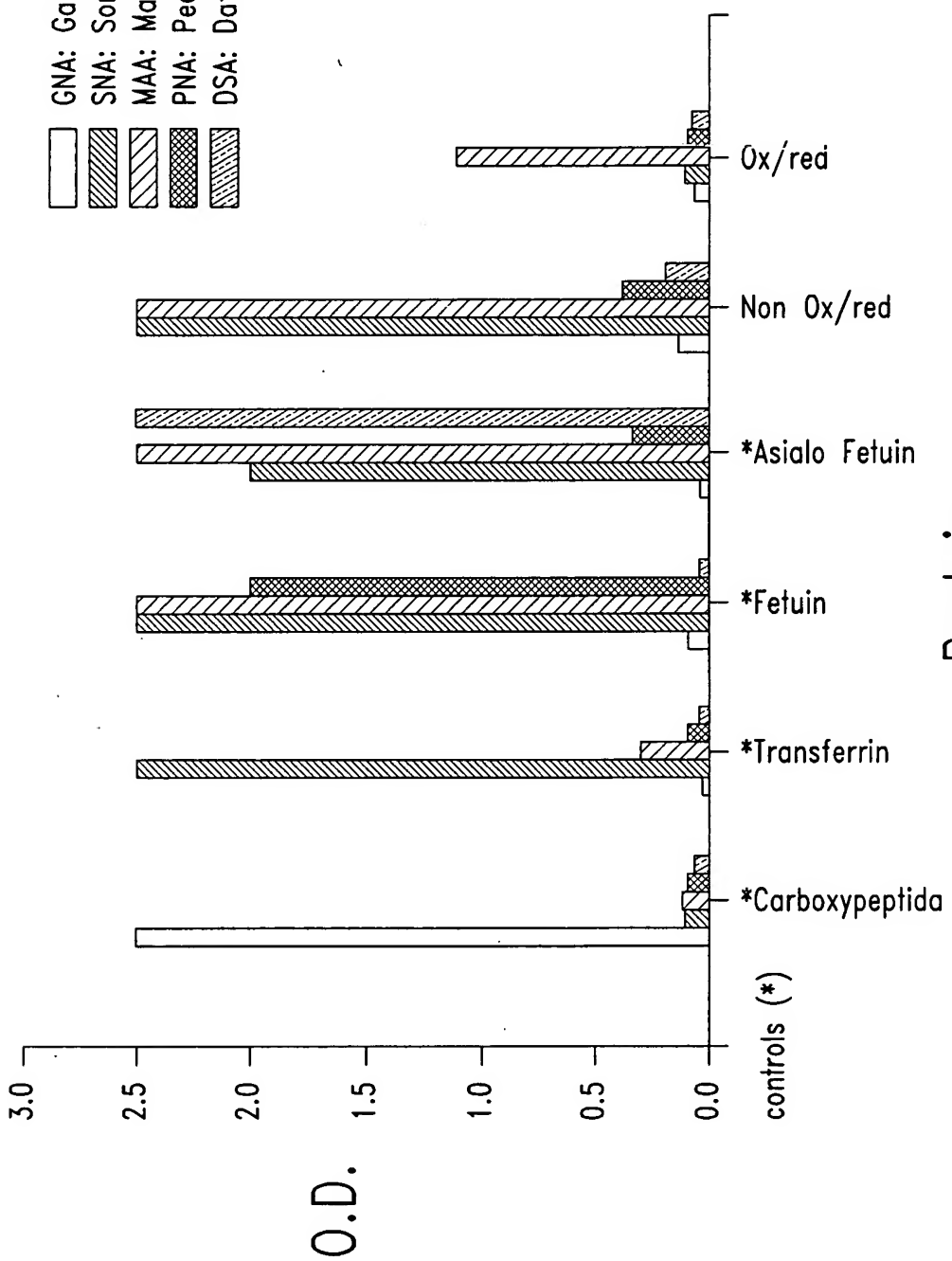
Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY

-LU-13 AND THEIR USE IN PRETARGETING METH

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2



- GNA: Galanthus nivalis agglutinin
- SNA: Sambucus nigra agglutinin
- MAA: Maackia amurensis agglutinin
- PNA: Peanut agglutinin
- DSA: Datura stramonium agglutinin



Protein

Fig. 14

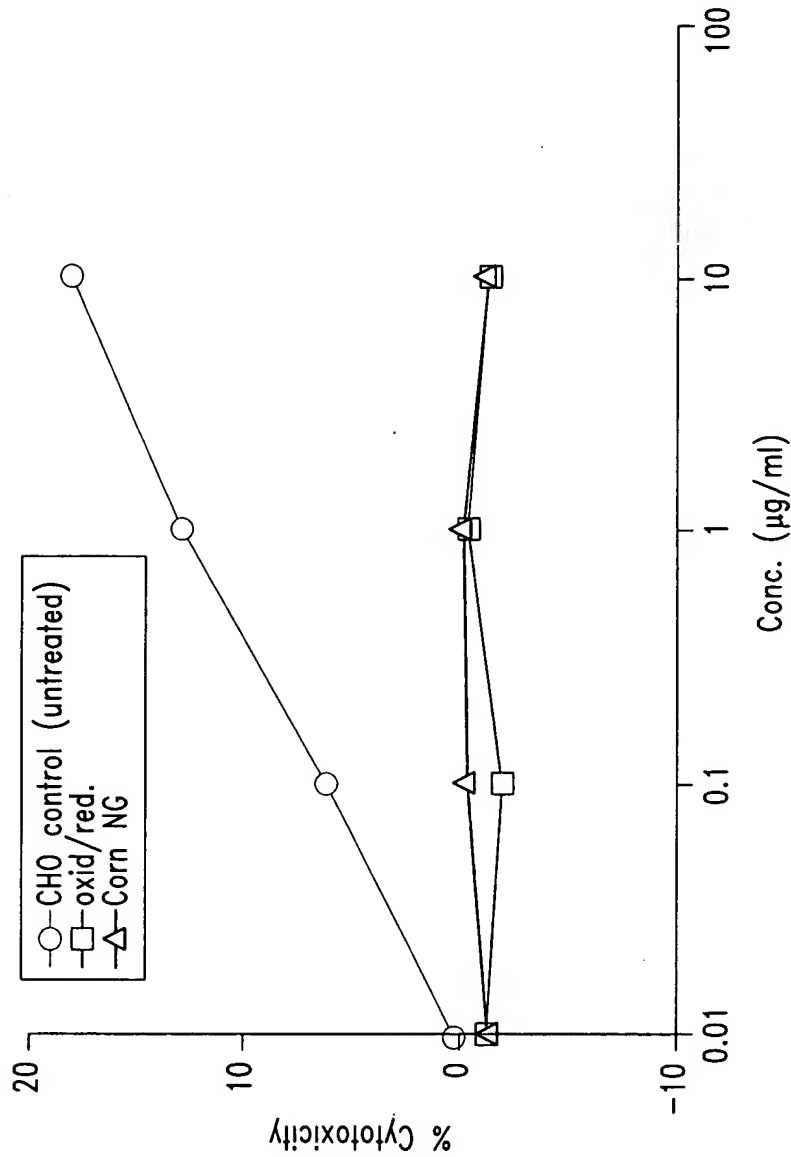


Fig. 15A

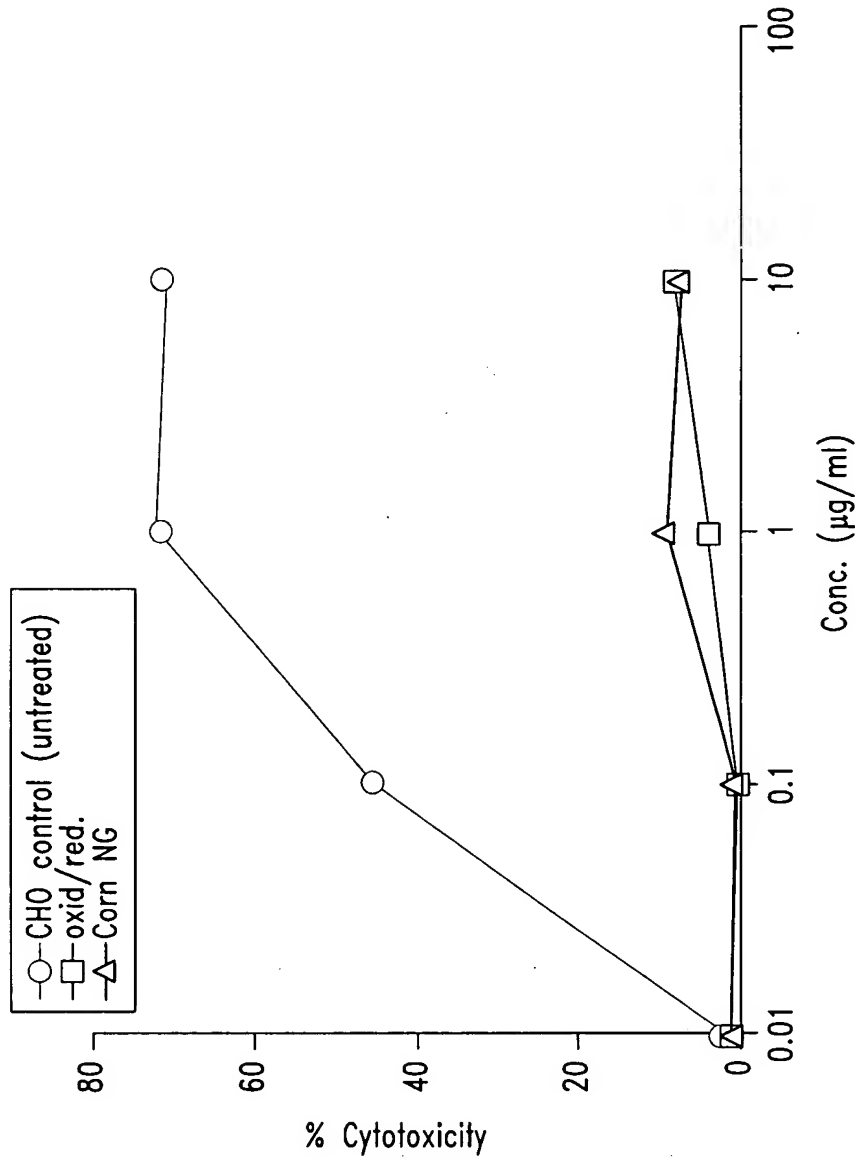


Fig. 15B

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY

-LU-13 AND THEIR USE IN PRETARGETING METH

Inventor(s): Scott S. Graves et al.

Serial No. 10/056,794

Docket No. 690022.527C2

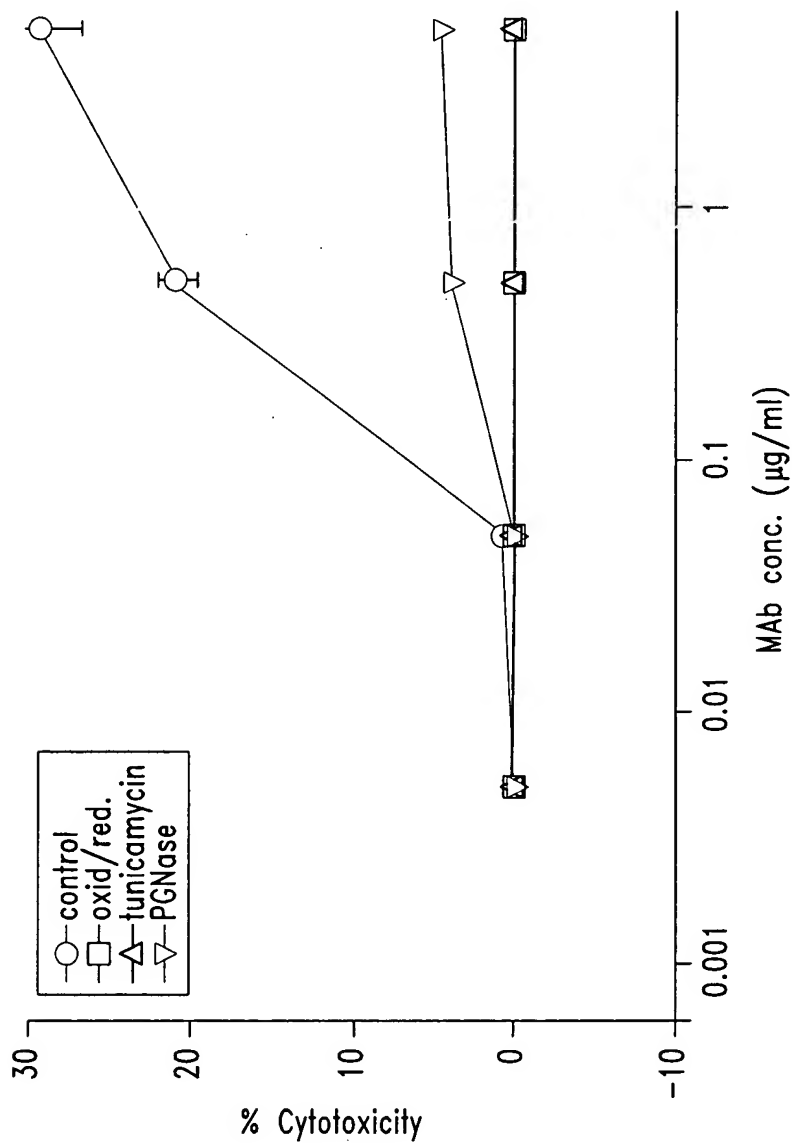


Fig. 15C

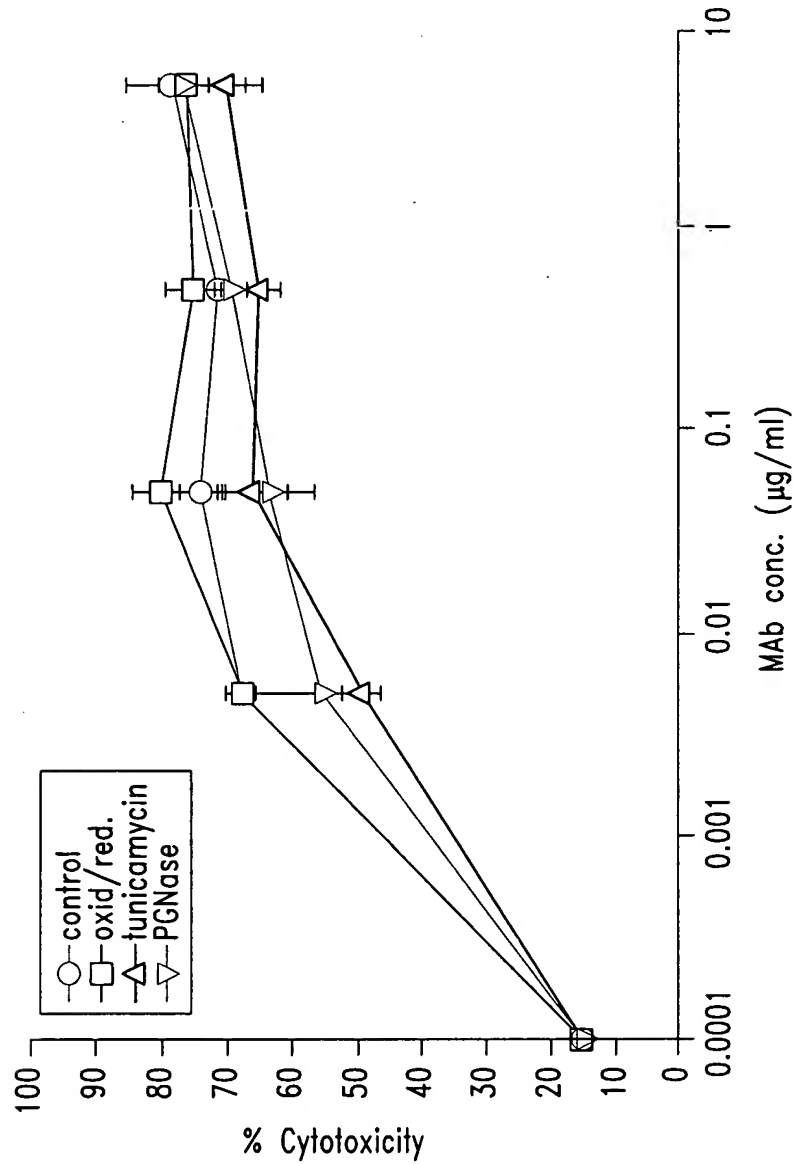


Fig. 15D

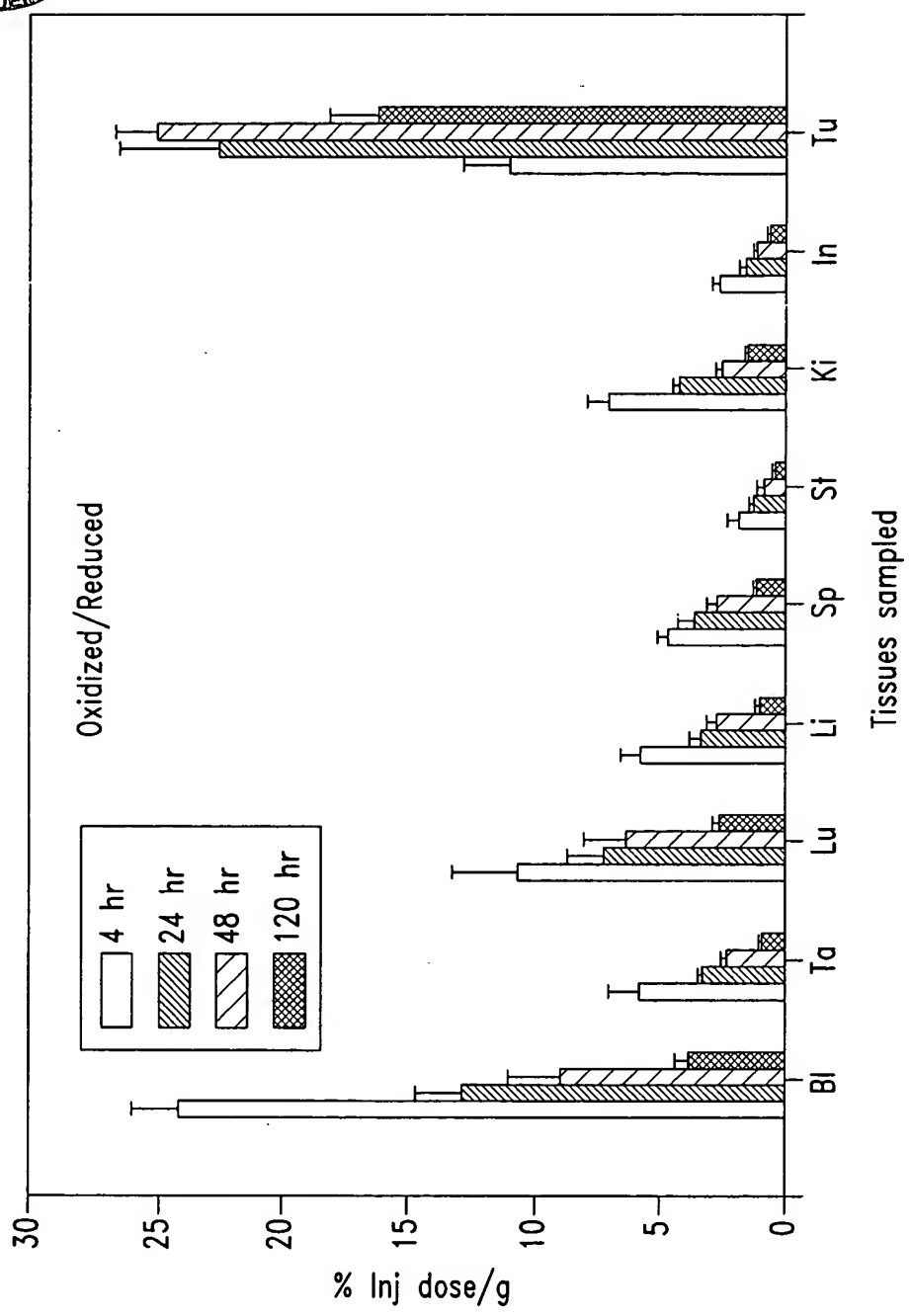


Fig. 16A

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY

-LU-13 AND THEIR USE IN PRETARGETING METH

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

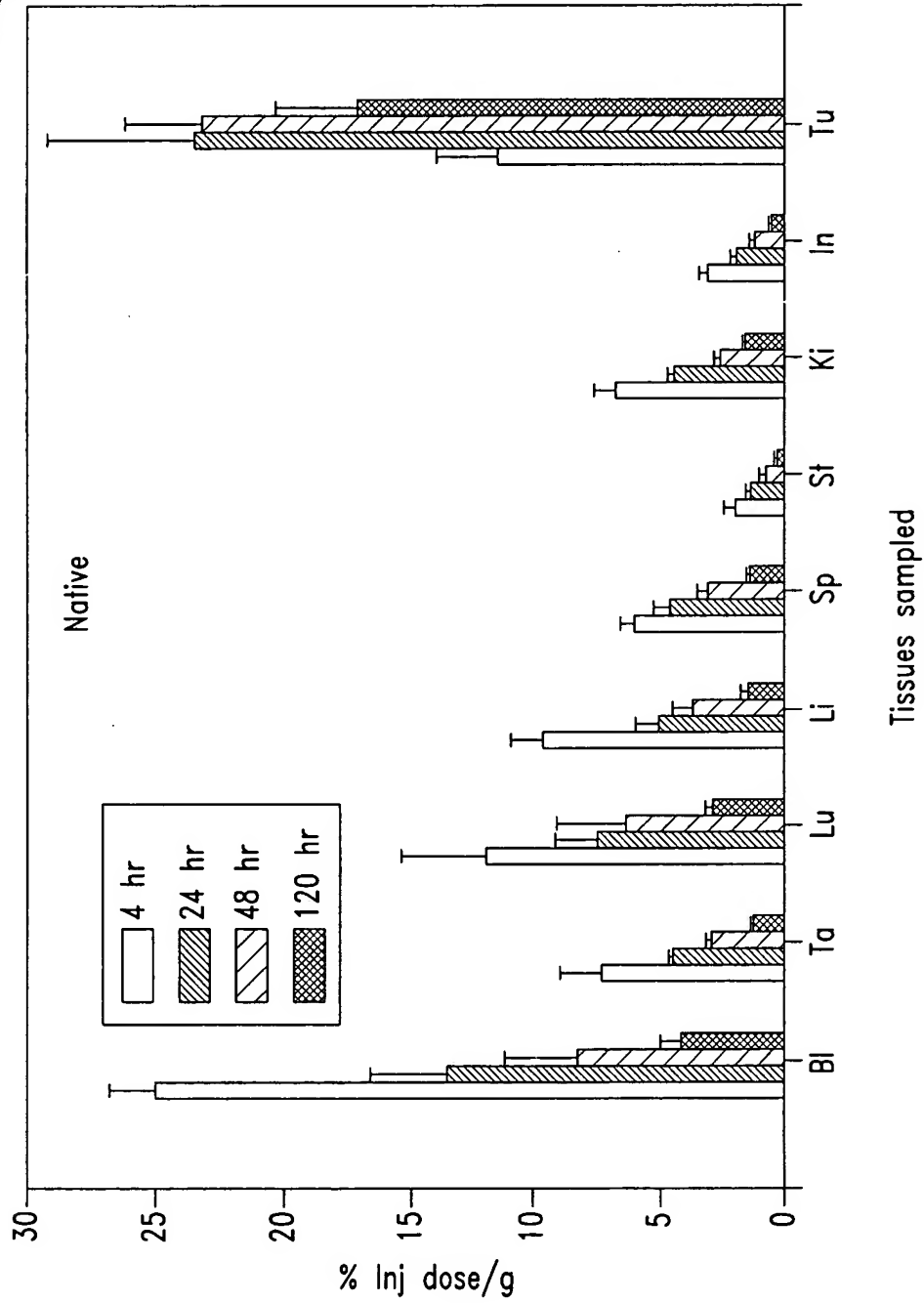


Fig. 16B

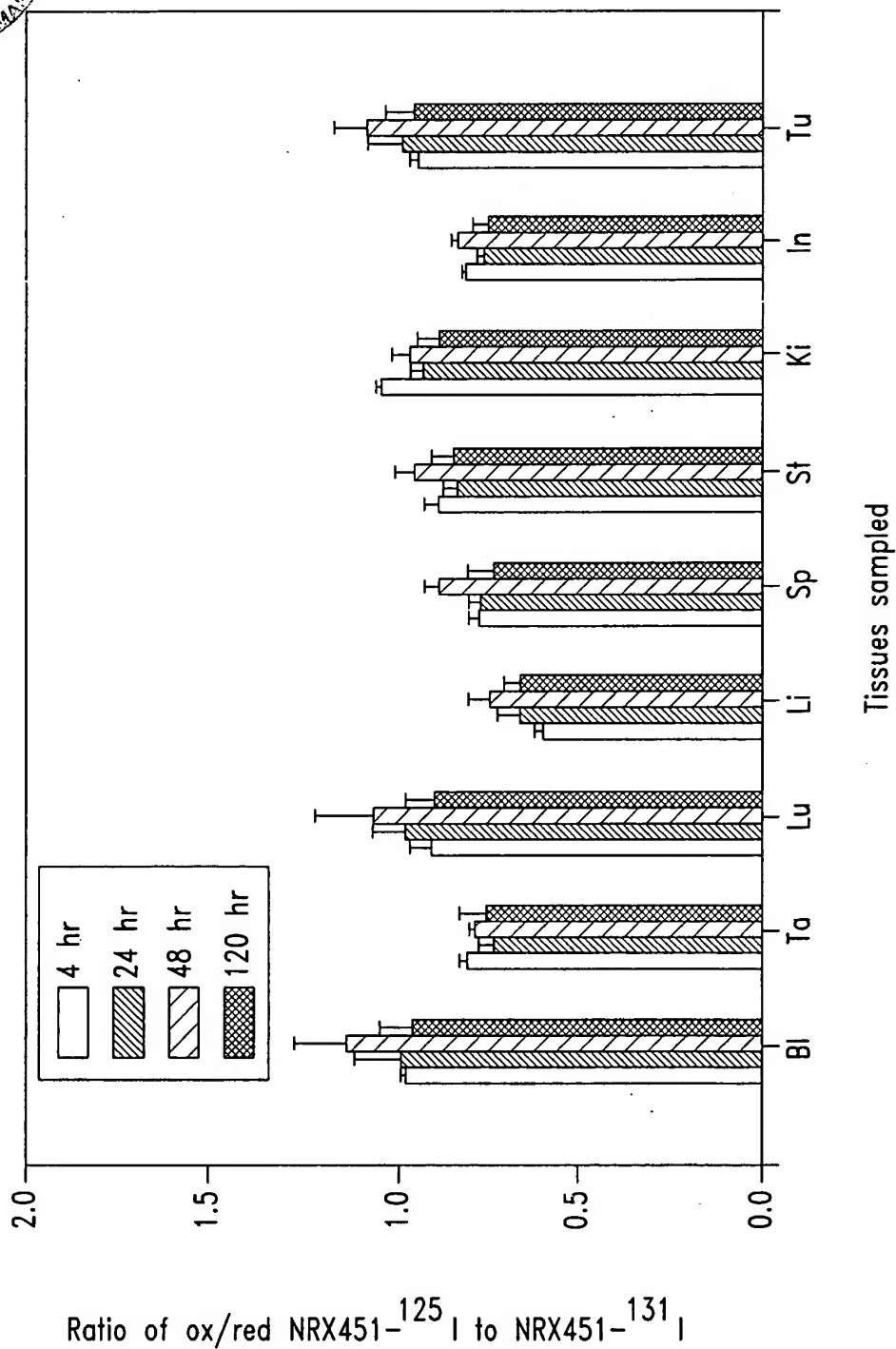


Fig. 16C

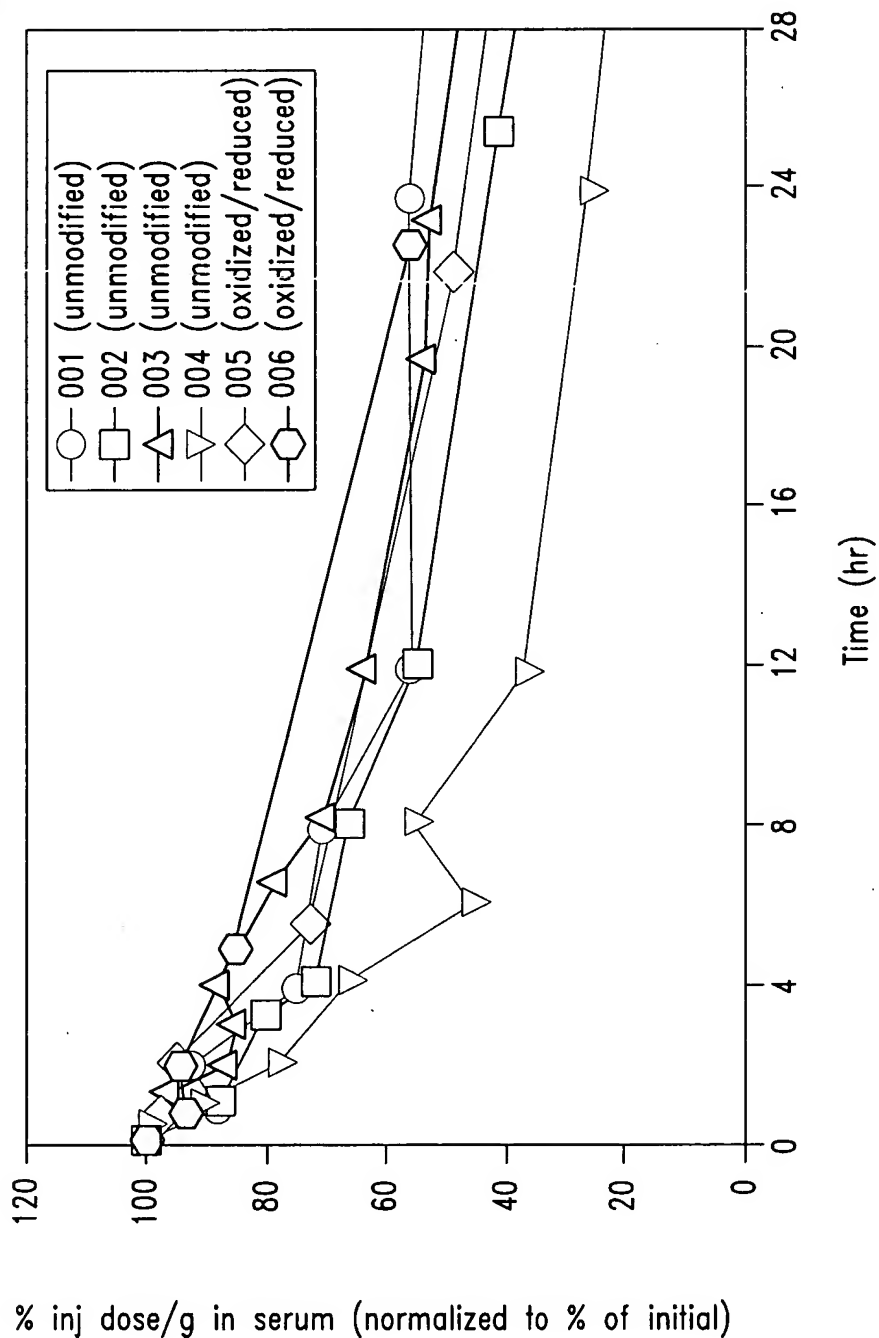


Fig. 17

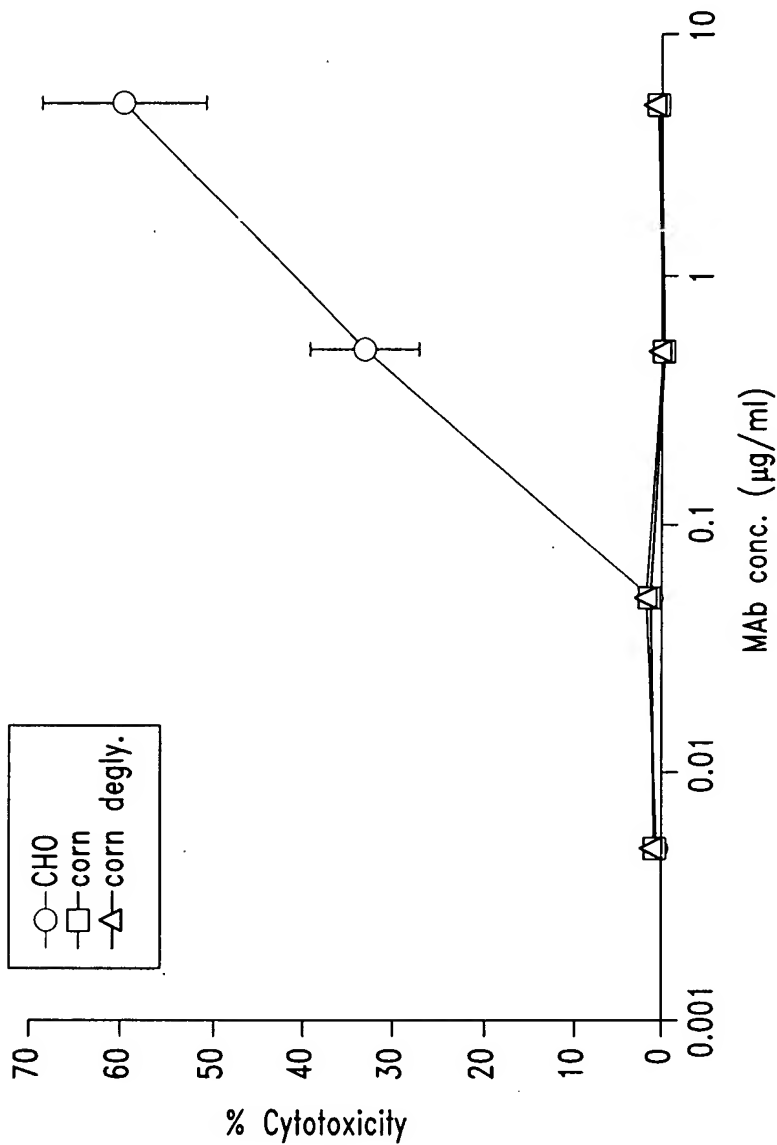


Fig. 18

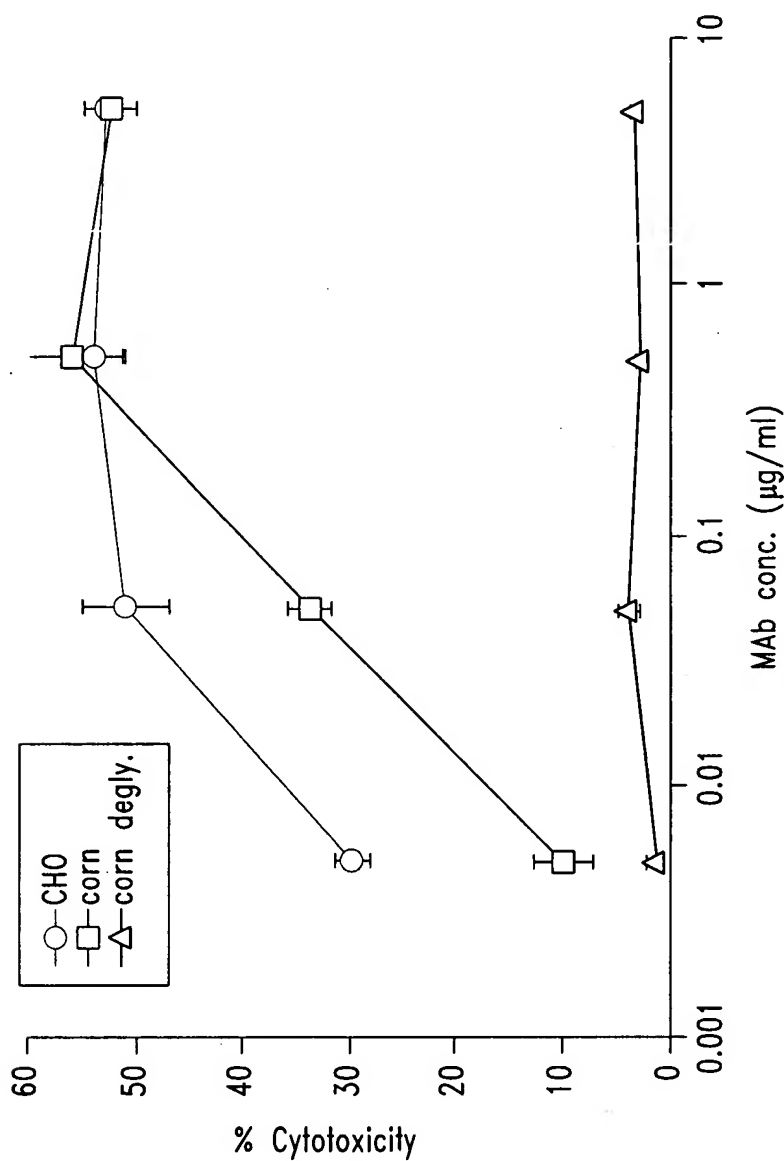


Fig. 19

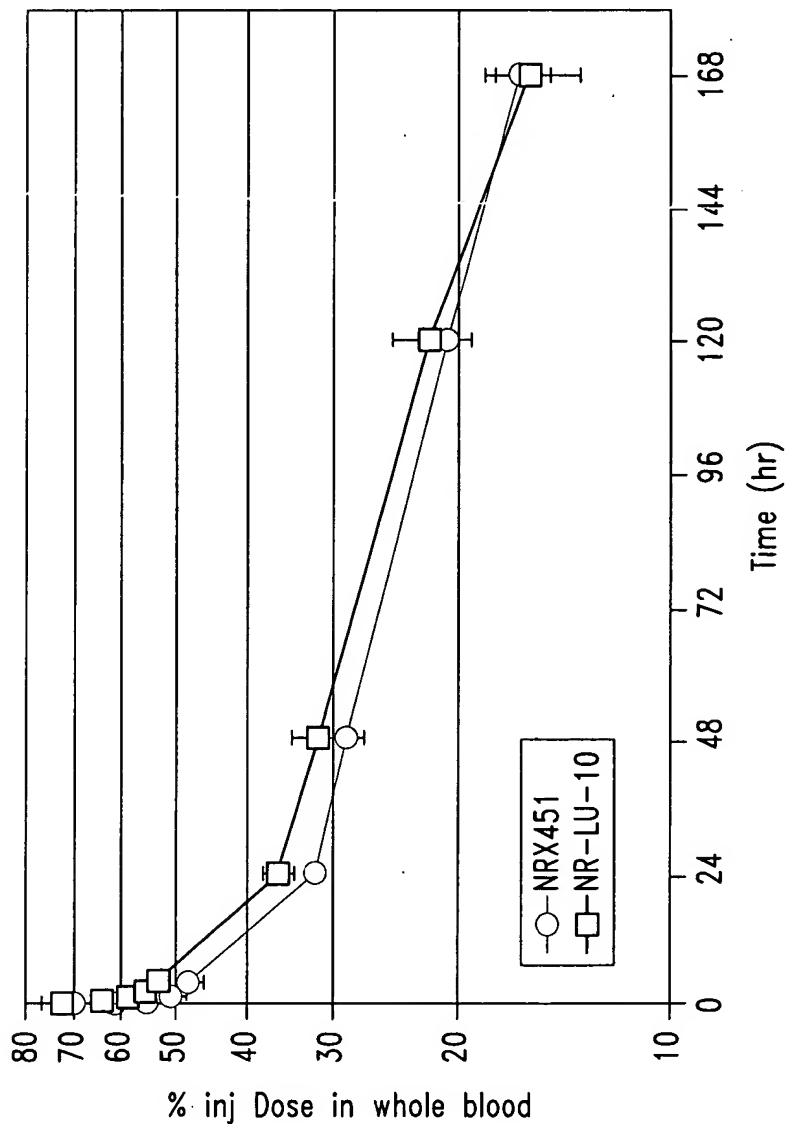


Fig. 20

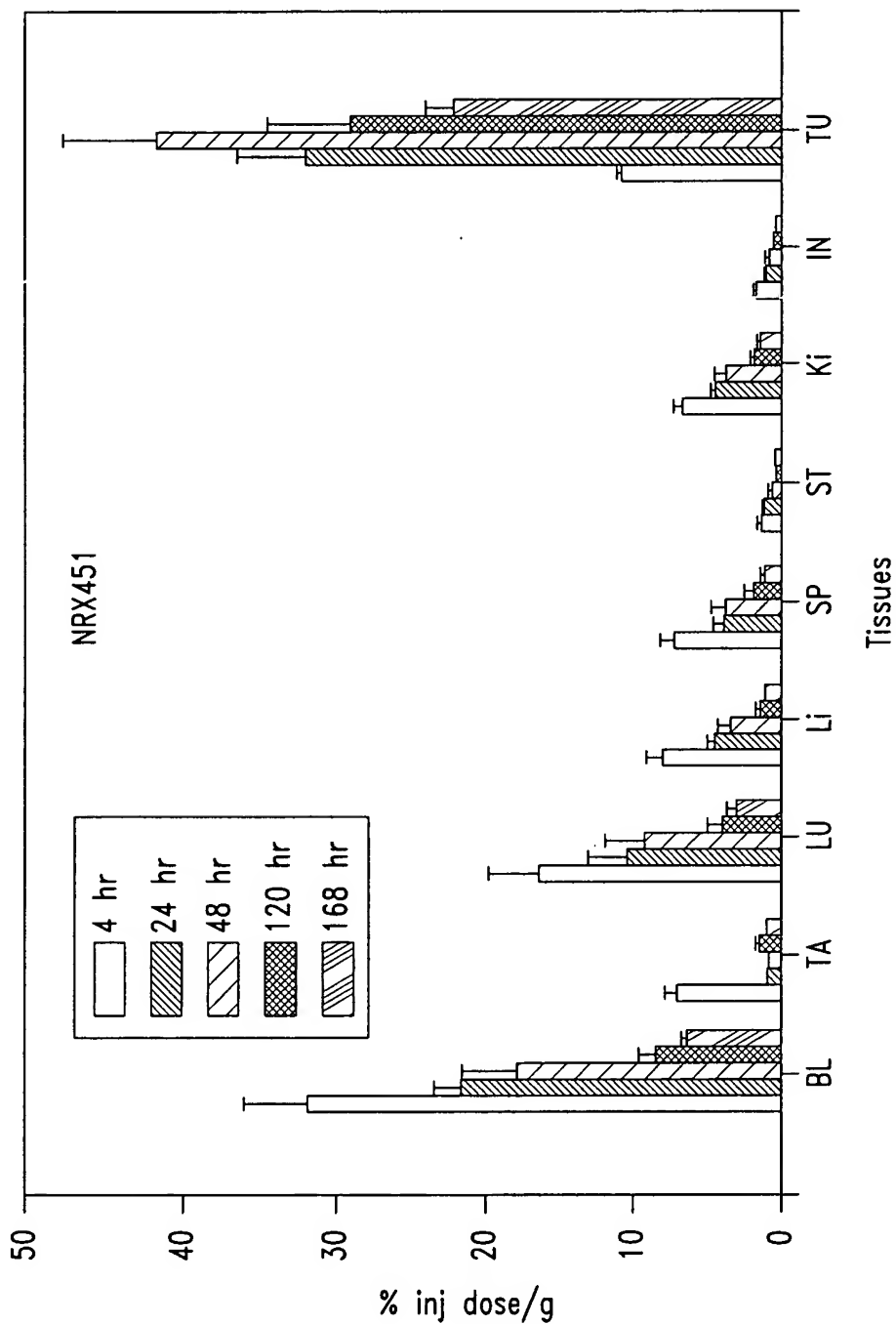


Fig. 21A

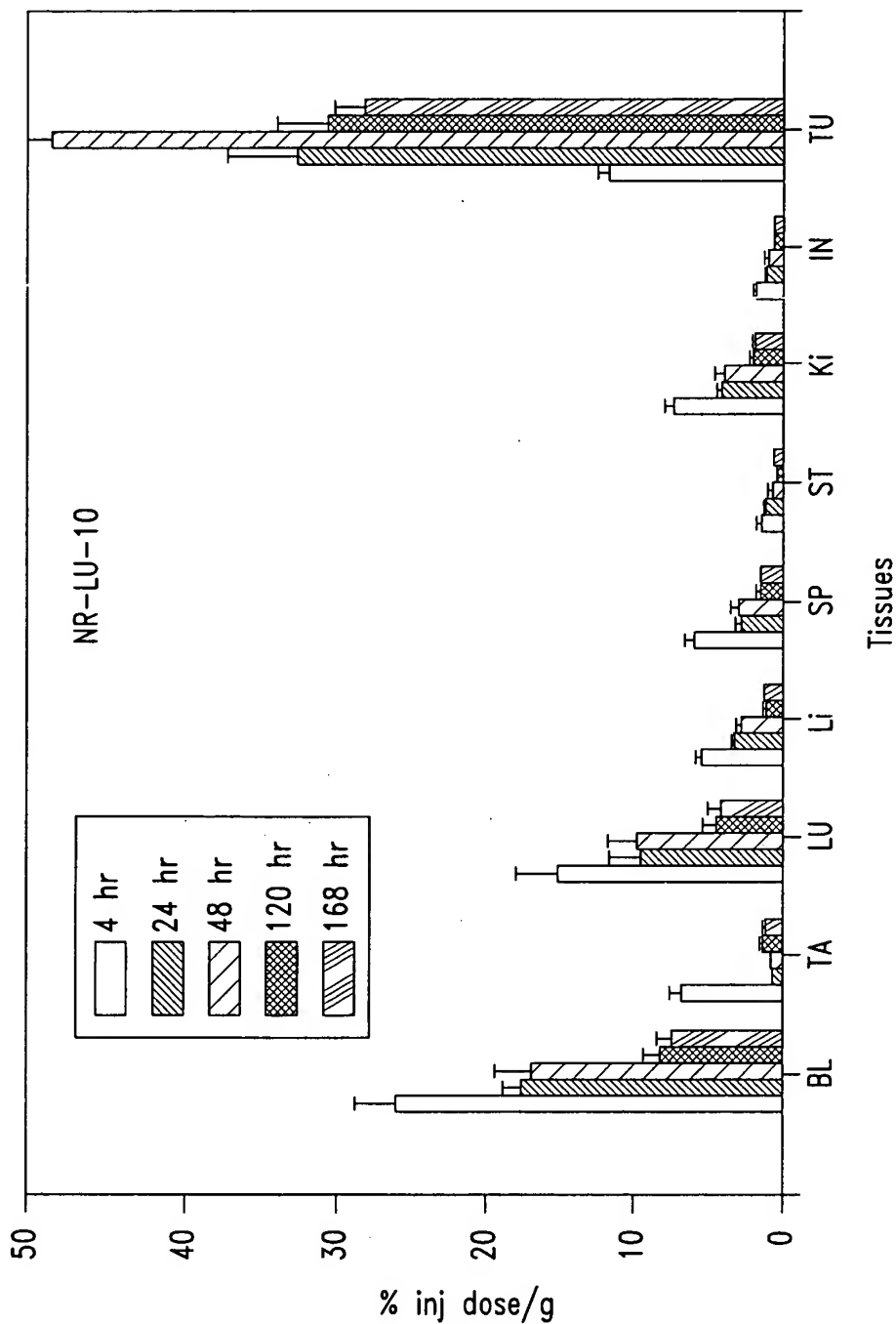


Fig. 21B

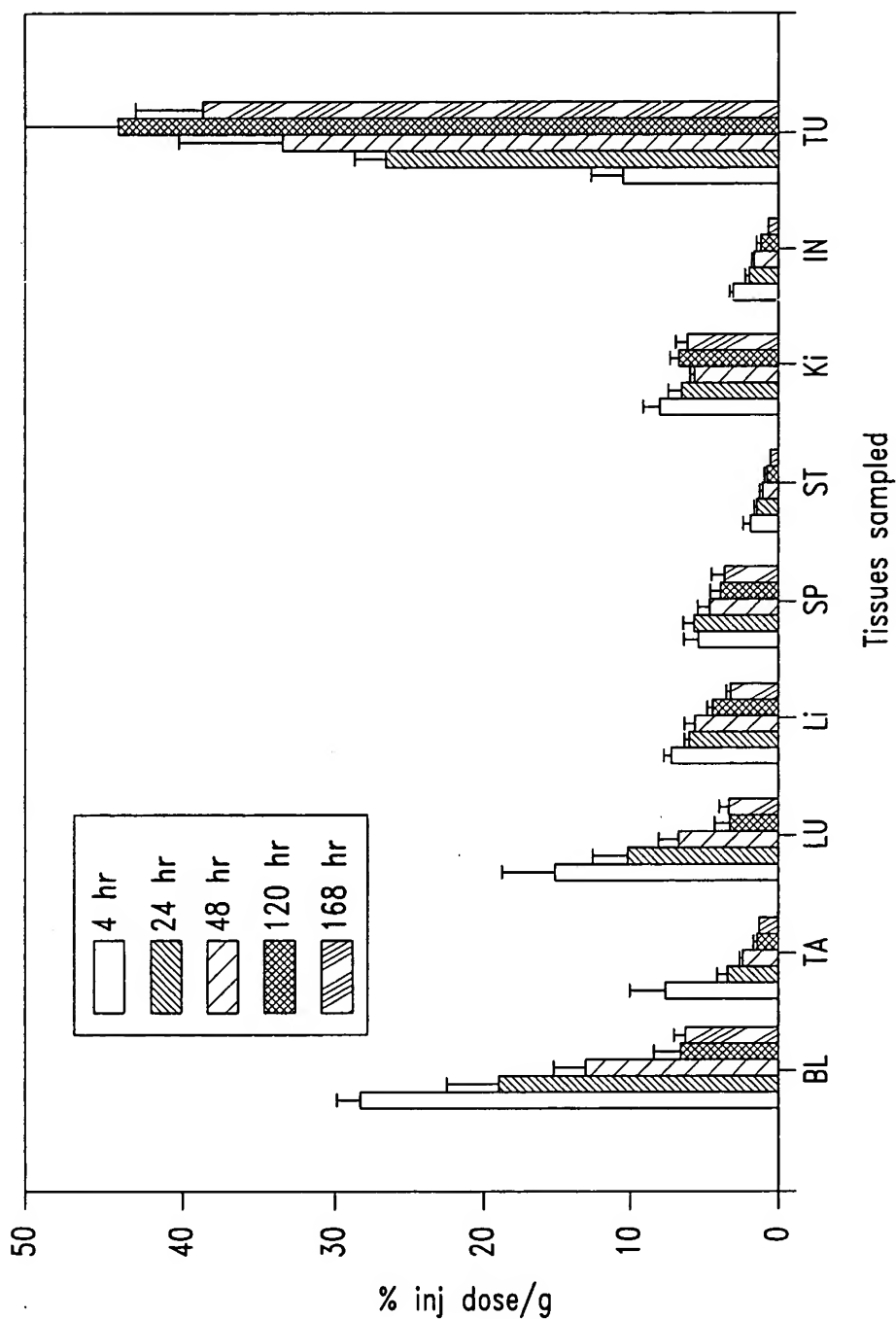


Fig. 22

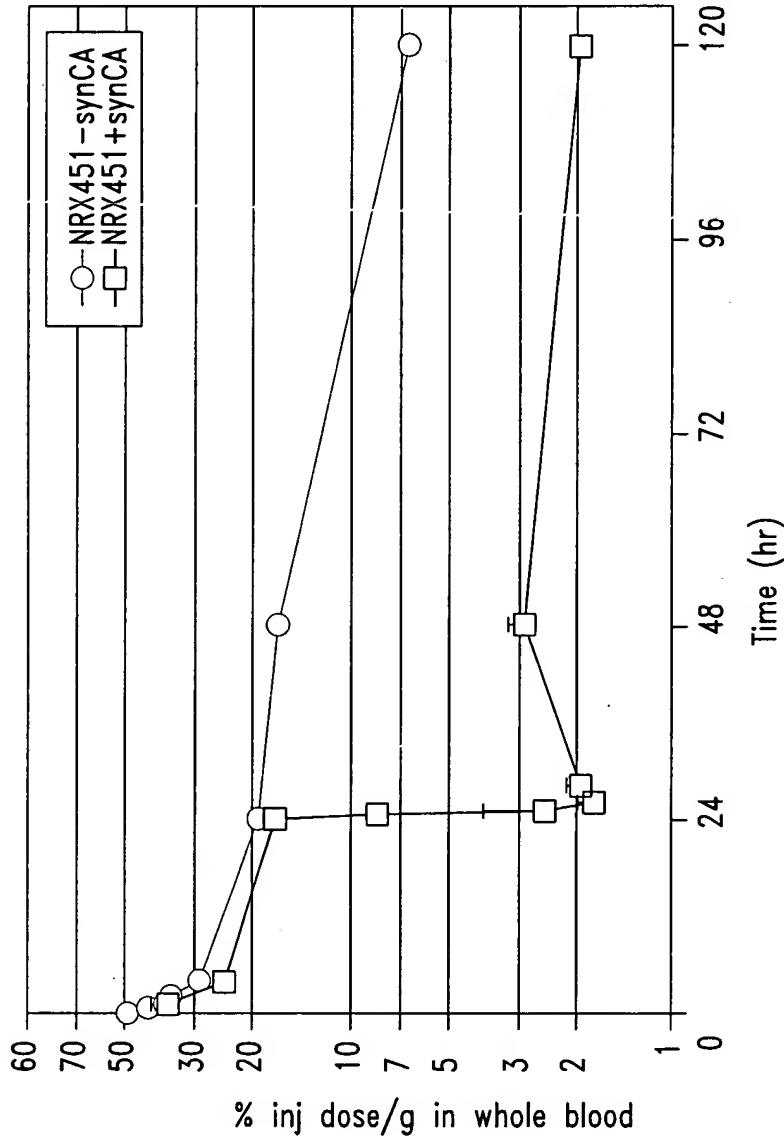


Fig. 23

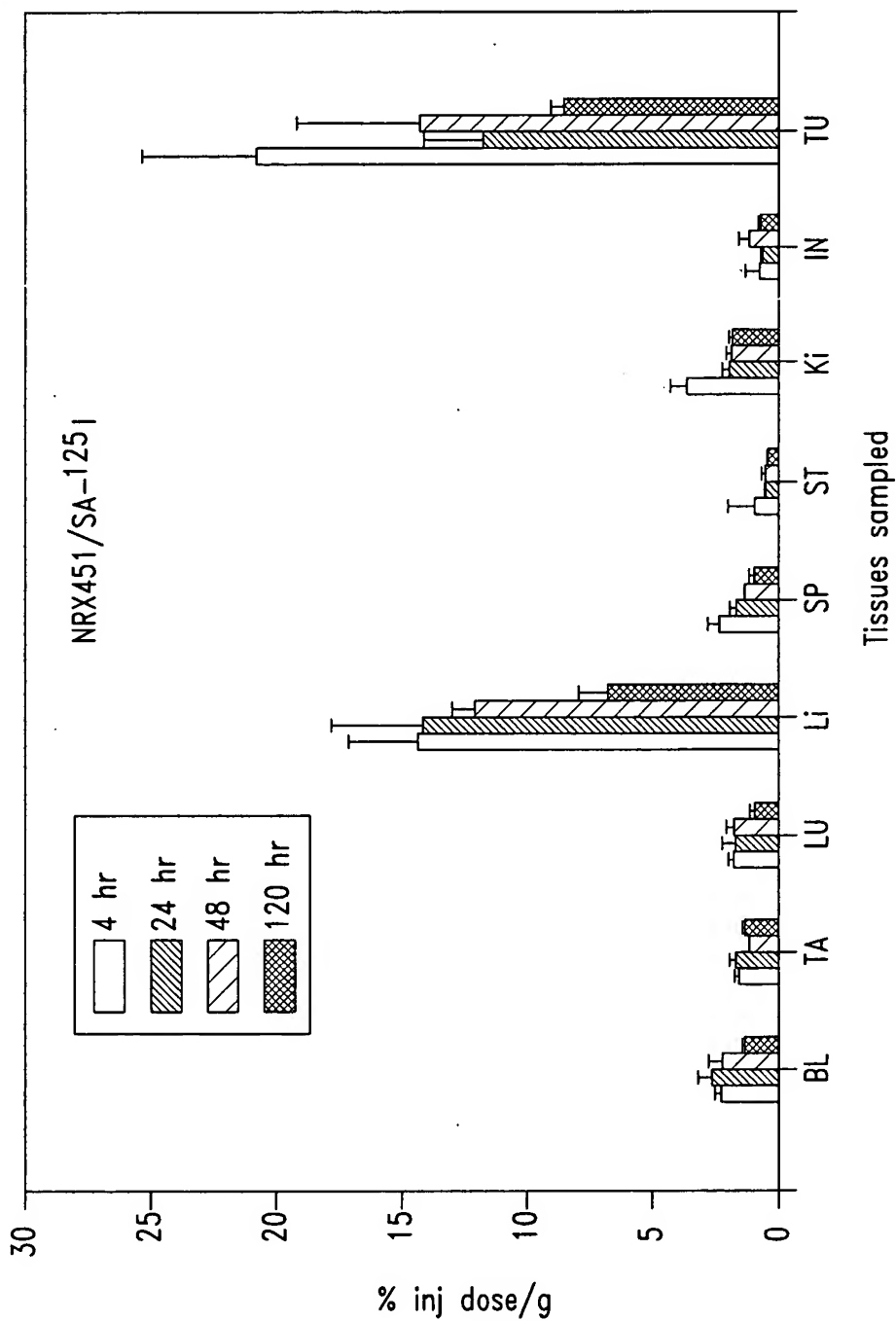


Fig. 24A

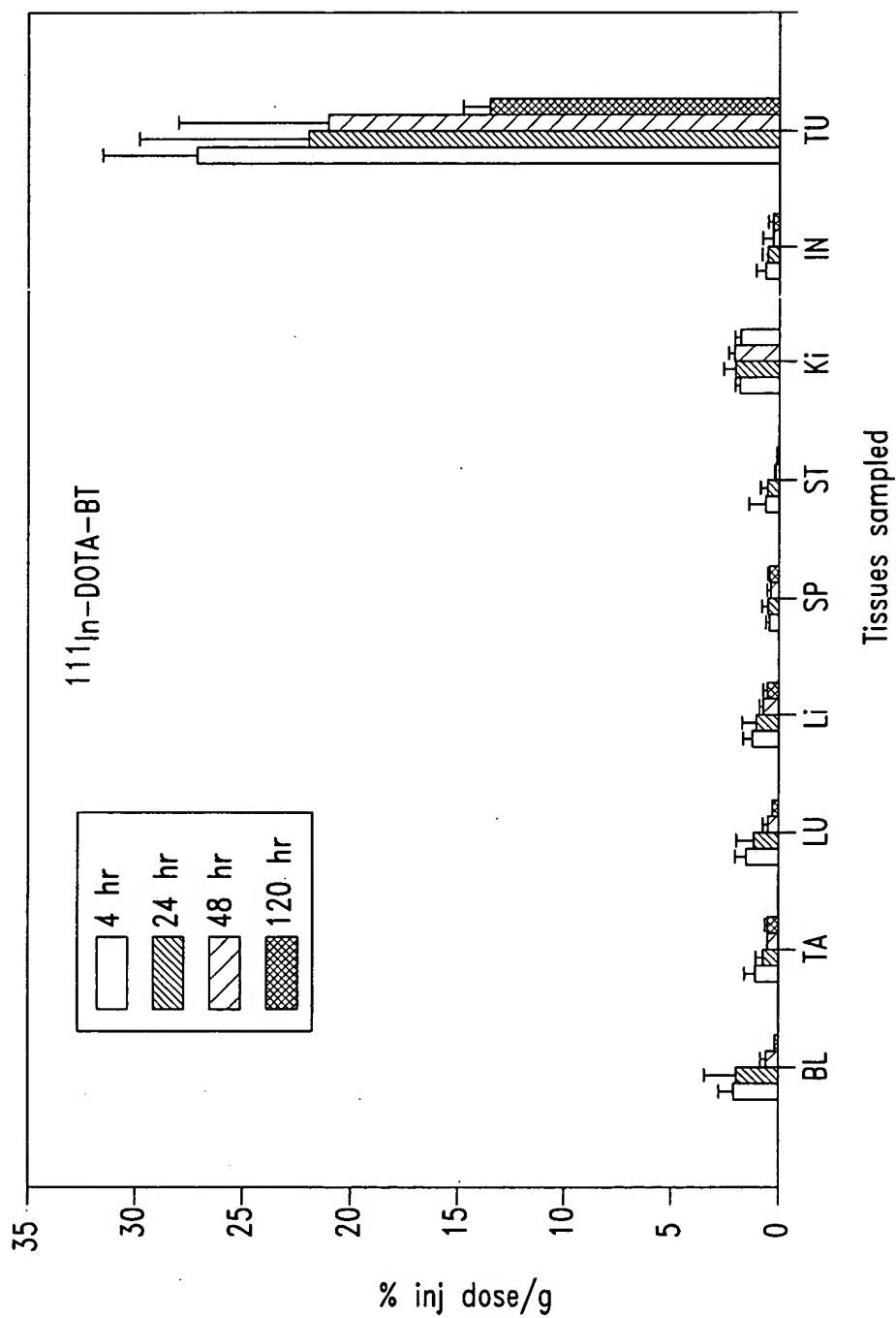


Fig. 24B